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ALTERATIONS IN GUT TRANSPORT OF MINERALS AND IN BINDING PROTEINS

DURING SIMULATED WEIGHTLESSNESS: NAGW-236

Final Technical Report

**(NASA-CR-175637) ALTERATIONS IN GUT
TRANSPORT OF MINERALS AND IN BINDING
PROTEINS DURING SIMULATED WEIGHTLESSNESS
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Period Covered: 08/01/81 - 11/01/84

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Justification

The structural components of the skeleton have developed and are maintained in a 1 g environment, shaped in part by the mechanical load to which they are constantly exposed. Altering such a mechanical load by reducing the gravitational force imposed on the system, as in space flight, has profound effects on the skeleton and permits an exploration of the molecular events which regulate normal skeletal homeostasis.

Objectives

At the onset of this project, the primary objective was to determine whether simulated weightlessness reduced intestinal calcium transport, and if so, to determine the molecular mechanisms for such an effect. A nonstressful tail suspension in which the rats gained weight normally while suspended was used to simulate weightlessness. Using the model, we could not demonstrate a significant change in intestinal calcium transport. However, we did demonstrate a cyclic change in bone formation with suspension. Based on these observations, our objective changed to determining the hormonal regulation of bone formation during simulated weightlessness. This work is continuing in our new grant with NASA-Ames Research Center (NCC 2 328).

Accomplishments

We have suspended growing rats (160-200g) by their tails for at least 4 weeks without altering their growth patterns. No difference in weight gain between such animals and their pair fed controls is observed. The suspended rats in comparison to the pair fed controls show a decrease in fat free weight, ash weight and calcium content of the unloaded bones (tibia, lumbar vertebra) but not of the normally loaded bones (humerus and mandible) (fig. 1). Most of the difference in bone mass between suspended and pair fed controls occurs progressively between days 2 to 10, after which no further difference between suspended and pair fed controls is observed. In the experiment depicted in figure 1 all rats were killed at the same age; the differences observed were the result of suspending rats at different times (and, thus, age) prior to sacrifice. We also suspended rats of the same age for differing periods of time (up to 4 weeks) and compared their bones with those of pair fed controls. These results (fig. 2) demonstrate that tibial bone mass increases linearly with time in the pair fed controls. In contrast, tibial bone mass did not increase between the 1st and 2nd week of suspension, but resumed a rate of growth parallel to that in controls between the 2nd and 4th weeks. The humerus of the suspended rats did not show this cessation of bone growth.

We examined the effect of suspension on two biochemical measures of bone formation: ^{45}Ca uptake presumably into mineral and ^3H -proline uptake presumably into matrix. In the experiment depicted in figure 3 and table 1 we separated the long bones into the proximal third (labelled metaphysis) and the shaft (labelled diaphysis) to determine whether suspension altered these regions of bone differently. As anticipated, the proximal portion of the long bones, which contains a higher percentage of endosteal bone than the shaft (which is primarily cortical bone and has a higher percentage of calcium/mg fat free weight) had a three fold greater amount of ^{45}Ca and ^3H -proline incorporation (table 1). With suspension both portions of the tibia showed a decrement in calcium content (fig 3A), calcium uptake (fig. 3B), and proline incorporation (fig. 3C) by 5 to 7 days when compared to the tibia from pair fed controls. After 10 days no further decrement in calcium content was seen and

^{45}Ca and ^3H -proline uptake rebounded to control or above control levels. The bone formation rate was determined at the tibia-fibula junction (cortical bone) in suspended rats and pair fed controls using three tetracycline labels administered on days 3, 9, and 14 to mark two periods of bone formation: period 1 = days 3 - 9, period 2 = days 9 - 14. The results (table 2) indicate that suspension leads to a 50% reduction in bone formation in the tibia during period 1, but with continued suspension these measurements of bone growth return toward normal.

Our initial approach to determining whether the calciotropic hormones were involved in signaling the unloaded bone to stop growing was to measure calcium, phosphorus, parathyroid hormone and $1,25(\text{OH})_2\text{D}$ in the serum of suspended and pair fed control rats (table 3). The results indicate no differences in the serum levels of any of these ions or hormones at 15 days. However, small increases in serum calcium were observed at days 5 to 7 of suspension which return to pair fed control values by day 15 (fig 4). The rise in serum calcium was associated with a fall in serum $1,25(\text{OH})_2\text{D}$ values (fig. 5). Furthermore, a small and usually insignificant reduction in intestinal calcium transport (a process regulated by $1,25(\text{OH})_2\text{D}$) occurs between 5-10 days of suspension, but calcium transport returns to control levels by day 15 (fig. 6). These observations suggest that changes in the serum levels of the calciotropic hormones during skeletal unloading may provide at least part of the mechanism by which bone formation is inhibited by day 5 and is restored to normal by day 10.

In an initial effort to prevent the deleterious effect of suspension on bone mass we determined whether increments in dietary calcium could prevent or lessen the inhibition of bone formation caused by suspension. As seen in figure 7, increasing dietary calcium from 0.1 to 2.4% markedly increased the calcium content of all bones, weighted or unweighted. However, the unweighted bones (tibia, L-1) in the suspended animals had a lower calcium content at all levels of dietary calcium above 0.1%. Histomorphometric analysis of these bones suggests that increasing dietary calcium reduces bone resorption with little effect on bone formation; the unweighted bones from suspended animals have a lower rate of bone formation than pair fed controls regardless of dietary calcium.

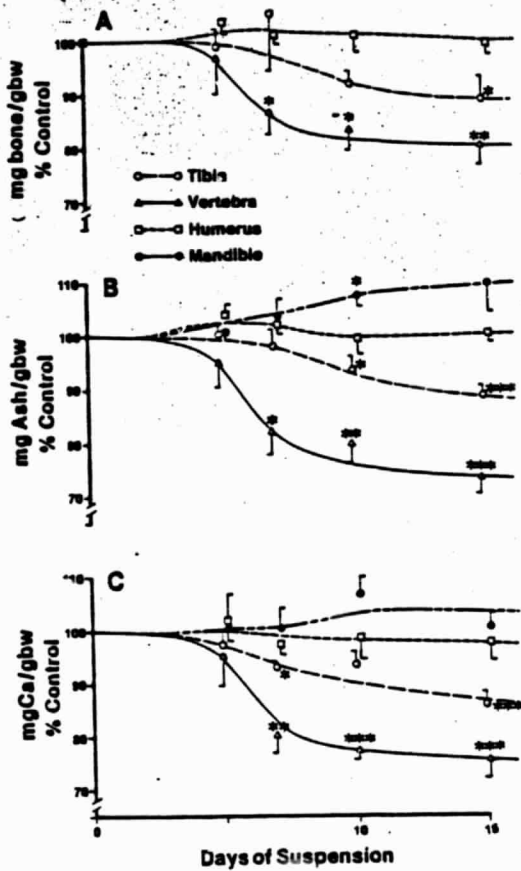


FIGURE 1

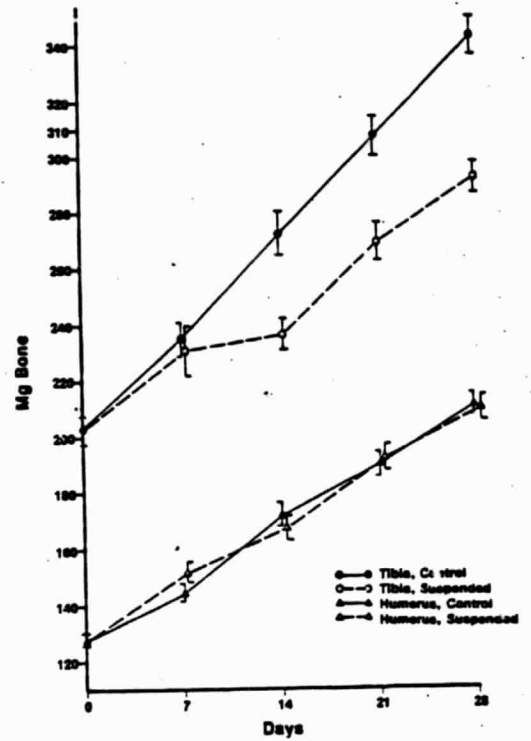
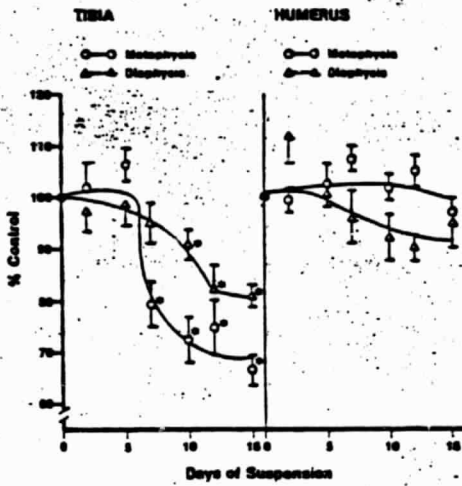
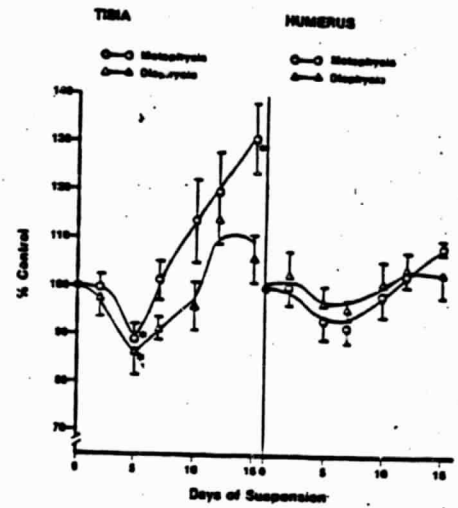


FIGURE 2

A



B



C

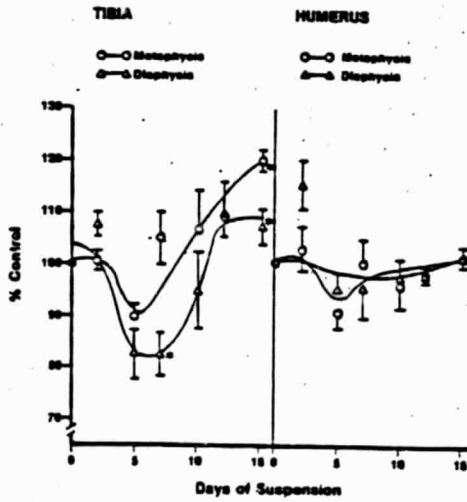


Figure 3 (a, b, c)

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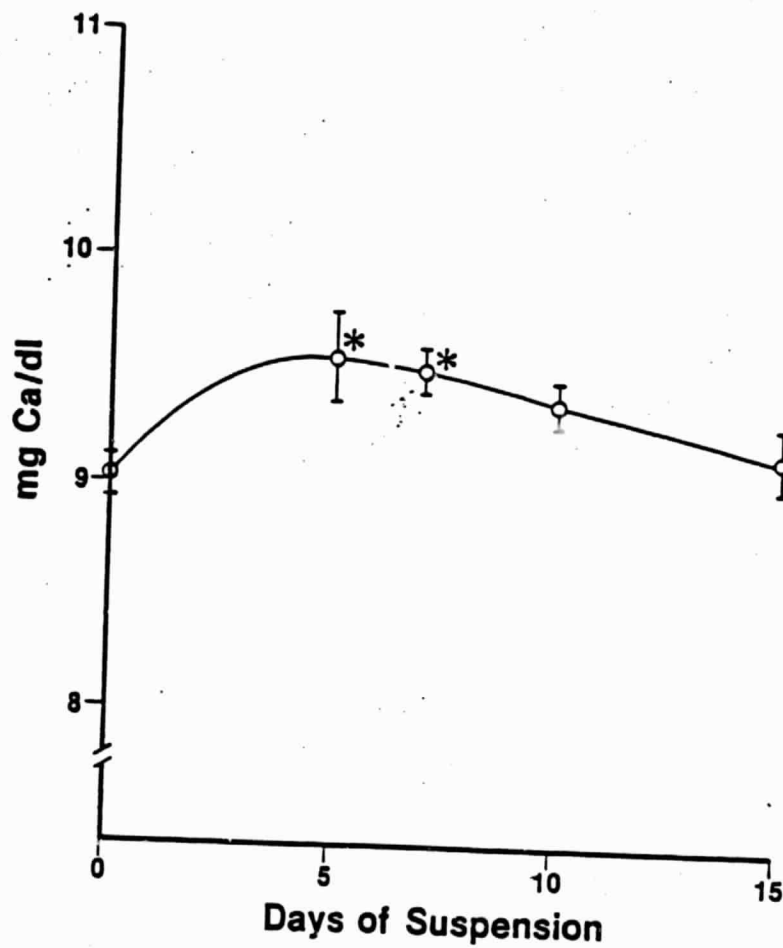


FIGURE 4

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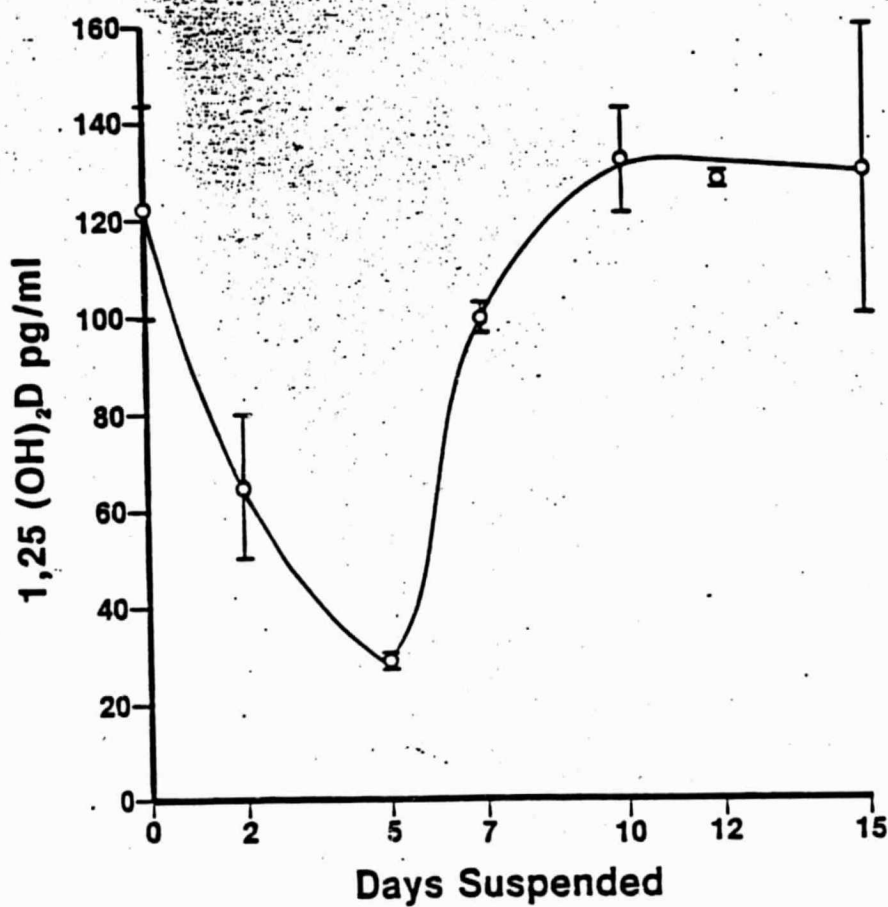


FIGURE 5

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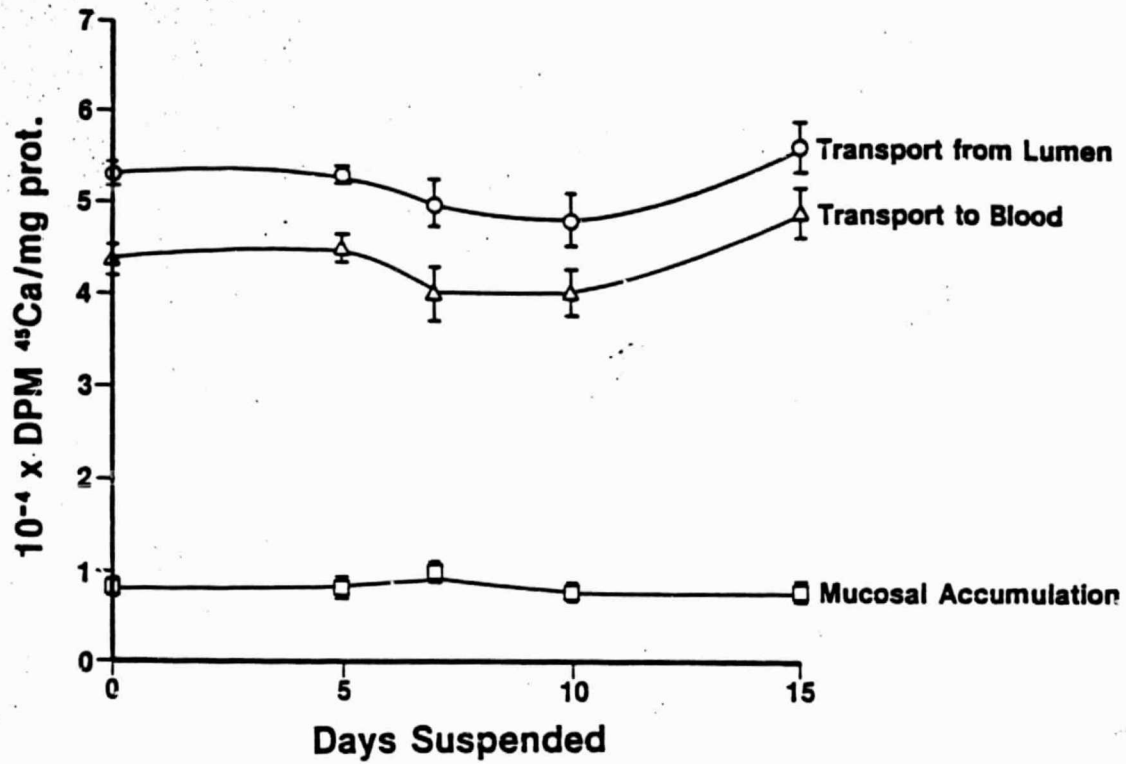


FIGURE 6

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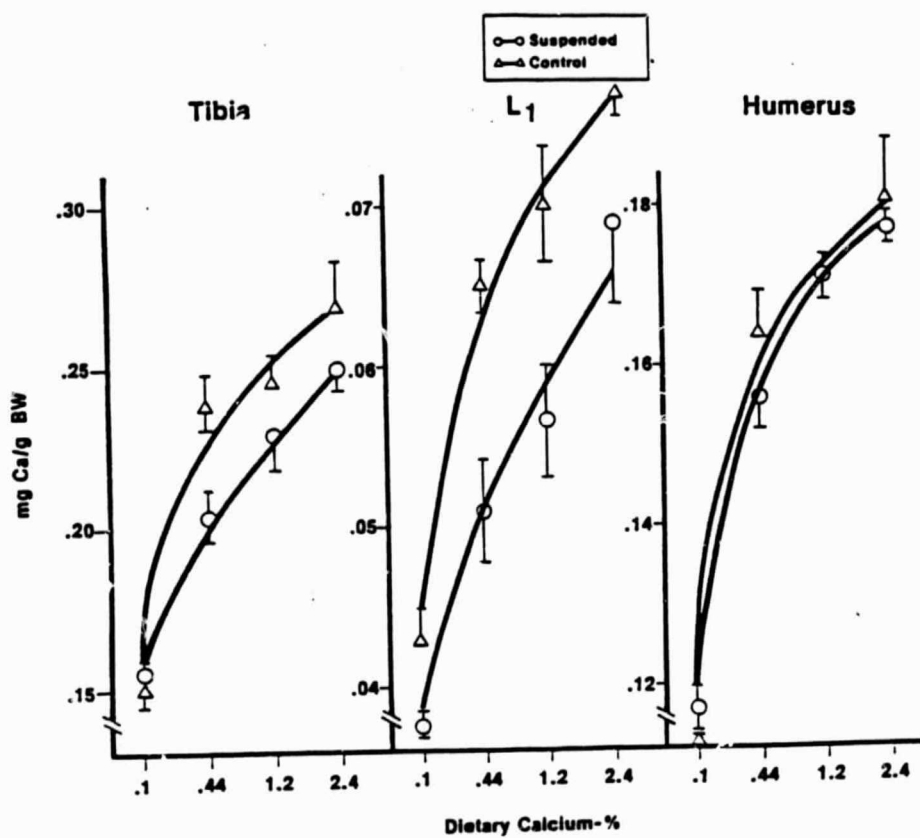


Figure 7

Table 1

Calcium Uptake, Proline Uptake and Bone Mass in Different Regions of Bone

	<u>Tibia</u>		<u>Humerus</u>	
	<u>Metaphysis</u>	<u>Diaphysis</u>	<u>Metaphysis</u>	<u>Diaphysis</u>
$^{45}\text{Ca}/\text{bone}^1$	215,800 \pm 7300	59,900 \pm 3000	138,200 \pm 5200	46,200 \pm 2000
$^3\text{H-pro}/\text{bone}^1$	116,000 \pm 8700	33,600 \pm 2300	69,800 \pm 4900	23,500 \pm 1700
mg Ca/bone ²	20.4 \pm 0.4	24.7 \pm 0.2	21.5 \pm 0.6	15.5 \pm 0.2
mg bone ³	124 \pm 4	116 \pm 3	111 \pm 3	69 \pm 4

1 = dpm per total bone fragment \pm SEM

2 = mg calcium per total bone fragment \pm SEM

3 = fat free weight of bone fragment \pm SEM

Table 2

Bone Formation Rate at the Tibia - Fibula Junction during the Initial (Days 3-9) and Later (Days 9-14) Periods of Suspension

	<u>Days 3 - 9</u>	<u>Days 9 - 14</u>
Suspended	0.019 \pm 0.003 ^{1*}	0.030 \pm 0.005 ^{*+}
Control	0.038 \pm 0.003	0.041 \pm 0.005

¹ = mean mm² \pm SD

* = p < 0.05 compared to control

+ = p < 0.05 compared to initial period

Table 3

Serum Calcium, Phosphorus, PTH and $1,25(\text{OH})_2\text{D}$
in Rats Suspended for Two Weeks and in their Pair-fed Controls

	<u>Suspended</u>	<u>Control</u>
Calcium ¹	10.2 ± 0.2	10.2 ± 0.1
Phosphorus ¹	6.2 ± 0.2	6.1 ± 0.1
PTH ²	0.65 ± 0.18	0.65 ± 0.1
$1,25(\text{OH})_2\text{D}^3$	168 ± 22	173 ± 28

1 = mg/dl

2 = ng eq/ml

3 = pg/ml

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